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OBSERVATIONS UPON CERTAIN PROPERTIES ACQUIRED BY THE PNEUMOCOCCUS IN THE HUMAN BODY.*

LEO BUERGER AND CHARLES RYTTENBERG.

(From the Pathological Laboratory, Mt. Sinai Hospital, New York.)

THE study of a large number of strains of pneumococci in the course of the last year's[1] routine bacteriological work led us to make certain observations upon the acquisition of new cultural properties by these organisms in the human body. In previous publications[2] one of us pointed out the variations that the pneumococcus may present in its morphology and in its cultural characteristics. The value of morphology in diagnosis was discussed as well as the value of the appearance of the colonies (ring colonies), the inulin and the glucose-serum media. Our experience led us to attach considerable importance to the typical morphology to which we applied the term "pneumococcus type." As regards the cultural properties it was found that although most pneumococci fermented inulin, certain streptococci also possessed this power, and that some pneumococci failed to break up this carbohydrate in every generation; further, that whereas streptococci caused precipitation in the glucose-serum-agar, only a very few pneumococci show this phenomenon; and finally, that streptococci usually produced hemolysis in blood media, but pneumococci either did not or only caused it in the blood in which they were found.[3] Thus, although not a single cultural property is differential between the streptococcus and pneumococcus, we may speak of a streptococcus and a pneumococcus cultural type, in order to facilitate future discussions. The streptococcus cultural type would include the following: non-fermentation of inulin, precipitation of glucose-serum-agar, and hemolysis in blood media; the pneumococcus cultural type, fermentation of inulin, non-precipitation, and failure to produce hemolysis.

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[1] 1906–1907.

[2] *Centralbl. f. Bakt.*, 1905, Orig. 39, pp. 216, 335, and *Jour. Exp. Med.*, 1905, 7, p. 545.

[3] LIBMAN, *Johns Hopkins Hospital Bulletin*, 1906, 17, p. 215.

While studying the blood of a case of puerperal pneumococcemia we isolated pneumococci differing from the ordinary type in that they failed to ferment inulin and caused precipitation of glucose-serum-agar (streptococcus cultural type). Upon further investigation we found that it was possible to convert this organism into the ordinary variety by animal inoculation. This led us to study carefully other pneumococci recovered from human exudates and blood, to investigate particularly those strains which varied from the type, and to attempt to effect a reversion to the normal by inoculation into susceptible animals. It is our purpose to record briefly a number of our observations.

OBSERVATIONS UPON CASE 1.

Puerperal pneumococcemia; streptococcus cultural type in three successive blood cultures and in a metastatic abscess; conversion into pneumococcus cultural type by passage through mice; failure to change properties of strain isolated from metastatic abscess.

1. Blood culture 863: March 18, 1907. Colonies on blood plates surrounded by zone of hemolysis. Morphology: chains with "streptococcus type" capsule. Inulin, negative;[1] precipitation,[2] positive.

2. Blood culture 870: March 21, 1907; same as above.

3. Blood culture 882: March 29, 1907; taken two days ante mortem. Colonies: some ring forms; hemolysis distinct. Morphology: like degenerate pneumococci; streptococcus type capsule. Inulin negative; precipitation positive.

Culture 9,729, from metastatic abscess in anterior and posterior chambers of eye. Morphology: typical pneumococci. Colonies: ring form. Inulin negative; precipitation positive.

Animal Experiments, April, 1907.

Culture 882*a:* in mouse 22, April 7; died in three days. Typical pneumococci in blood. 882*b* isolated.

Culture 882*b:* typical pneumococcus; inulin positive; precipitation negative.

Culture 882*a*, in mice 24 and 25; both died after 24 hours. 882*c* and 882*d* isolated.

Cultures 882*c* and 882*d:* typical pneumococcus morphology and cultural type.

Culture 9,729: in mice 23 and 28; mouse 23 killed in 5 days, negative; mouse 28 died in 2 days. 9,729*a* isolated; inulin negative; precipitation positive. Successive inoculations into mice; same result.

Animal Experiments, May, 1907.

Culture 882*a:* in mouse 48, May 7, died in 3 days; pneumococcus of typical morphology in blood. Streptococcus cultural type. Several other mice inoculated with same result. Hence the organism cannot now be converted to pneumococcus cultural type.

Culture 9,729 in a number of mice; remains streptococcus cultural type.

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[1] Negative means not fermented.

[2] Precipitation refers to whitening or precipitation in glucose-serum-agar.

Animal Experiments, June, 1907.

Cultures 882*a* and 9,729 into several mice on June 6 and June 24. The organisms remained of the streptococcus cultural type.

Here we have a significant instance of the labile nature of the pneumococcus and of the mutability of its fermentative properties. The organisms from the blood possessed the cultural features and even the morphology of a streptococcus. A metastatic focus contained organisms of the typical pneumococcus morphology but otherwise resembling streptococci. It is interesting to note that three of the strains reverted to the normal type after passage through white mice, but that one organism from the eye focus retained its peculiar properties. After the lapse of one month it was found impossible to bring about a change to the normal type in any of the cultures and even those that had been converted assumed their original features. After three months the streptococcus cultural type seems to have become fixed, not only in the original strains but also in those that had for a time taken on the usual cultural features of pneumococci.

OBSERVATIONS UPON CASE 2.

Chronic endocarditis, lobar pneumonia and pneumococcemia. Streptococcus cultural type; conversion into pneumococcus cultural type.

Organisms studied: (*a*) and (*b*) blood cultures; (*c*) postmortem, lung culture; (*d*) postmortem, spleen culture.

Culture (*a*) morphology typical pneumococcus; colonies large, white on plates, surrounded at first (24 hours) by green area, later by zone of hemolysis. Inulin negative; precipitation positive.

Cultures (*b*), (*c*), and (*d*), same as (*a*).

Culture (*b*) inoculated subcutaneously into two white mice; death in 24 and 48 hours respectively. Two strains isolated b_1 and b_2.

Strain b_1, pneumococcus cultural type.

Strain b_2, streptococcus cultural type.

In this instance we were dealing with pneumococci that had acquired the cultural features of streptococci in the human body. Passage through a white mouse effected a complete change as regards inulin fermentation and precipitation of glucose-serum-agar.

OBSERVATIONS ON CASE 3.

Acute osteomyelitis; pneumococcus with typical capsules and streptococcus cultural properties; it remained unchanged after repeated animal inoculation; a later culture obtained at a second operation showed typical pneumococci both as to morphology and cultural characteristics.

Culture (*a*) from subperiosteal exudate. Culture (*b*) from marrow cavity. Cultures identical. Morphology: a few diplococci and many short chains; they are of the pneumococcus type, but somewhat mucoid, inulin negative; precipitation positive.

Animal experiments.

Mouse 33, April 15, 1907; died in 48 hours. Cultural properties unchanged, still of streptococcus type. This strain was inoculated into mouse 35 and the organism isolated was again passed through mouse 37. No change in properties was thus effected. On June 6 and 24, all the strains were again tested and still found to possess the streptococcus cultural type.

Culture (*c*) isolated *two and one-half weeks later* at a second operation. *Typical pneumococcus*—both in morphology and culture.

Another variation different from the preceding presented itself to us in this case. The difference in the cultures obtained at the first and second operations are interesting. The first organisms isolated retained their streptococcus cultural properties despite successive animal inoculations whereas the second strain derived from the same source two and one-half weeks later was a typical pneumococcus.

OBSERVATIONS ON CASE 4.

Retropharyngeal abscess; pneumococcus of the mucoid type[1] *with typical morphology, but possessed of the streptococcus cultural type; failure to convert it into the pneumococcus cultural type by repeated animal inoculations.*

Culture 9,838: typical pneumococcus morphology; inulin negative; precipitation, positive.

Animal Experiments.

Mouse 31, inoculated April 11, 1907; remains of streptococcus cultural type. Mouse 36, April 23, ditto. Otherwise gave same result. June 6 and 24—animal inoculations; streptococcus cultural type persists.

This organism presents still another variety, namely a mucoid pneumococcus that rapidly lost its luxuriant growth, and that failed under any circumstances to ferment inulin. Such pneumococci are the most difficult to classify, for it is only by their morphology that they can be recognized. Possibly some bacteriologists would regard them as atypical streptococci rather than pneumococci. We are inclined to look upon this strain as one that had acquired new cultural features in the human body, and had retained its morphological characteristics.

[1] BUERGER, *Jour. Exp. Med.*, 1905, 7, p. 497.

In view of the fact that it was found possible to convert certain strains from the atypical into the typical variety by means of animal inoculation, it seemed of practical as well as of theoretical interest to determine whether the same result could be obtained by the use of favorable media; and, vice versa, to inquire into the action of human blood on the typical organisms with a view to seeing whether it would cause the same changes in the cultural features that were found to take place in the human body.

Pure sterile ascitic fluid exerted a marked effect upon certain strains; indeed, a 24-hour growth in this medium often produced large encapsulated forms which grew luxuriantly for a number of generations. However, the power to ferment inulin could not be restored by its use, and the influence of this medium was confined to a temporary enhancement of growth and a change in morphology.

A number of typical pneumococci were grown in sterile human blood in which coagulation had been prevented by an admixture of sterile ammonium oxalate solution 0.2 per cent. The blood was obtained by aspiration from one of the veins of the forearm, and about 3 to 5 c.c., mixed with an equal amount of ammonium oxalate, were used for each test. It had been previously found by Bernstein and Epstein[1] that the ammonium oxalate does not interfere with the growth of most of the pathogenic organisms. Subcultures were made after intervals of 3, 4, 5, and 7 days and the organisms were tested on inulin and glucose-serum-agar. It was not found feasible to convert a pneumococcus cultural type into the streptococcus variety by this means.

Another point which seemed worthy of investigation was the possibility of effecting a change in the cultural properties of atypical organisms after the lapse of several weeks or months. With this end in view the strains of the streptococcus cultural type were again passed through white mice after periods of about two and three months. Two months after isolation all the atypical organisms had apparently acquired fixed streptococcus cultural characteristics and repeated animal inoculation failed to bring about a change. The same negative result was obtained when the experiments were repeated one month later.

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[1] *Jour. Infect. Dis.*, 1906, 3, p. 772.

GENERAL SUMMARY.

Observations on the morphology and cultural features of pneumococci in human exudates and human blood lead us to the conclusion that wide variations from the type may occur. They tend to point rather significantly to the influence that the human body may exert upon the fermentative properties of these organisms. From a consideration of the variations from the type and from the results of animal experiments we must needs become impressed with the mutability of the fermentative properties of the pneumococcus and become even more careful in attaching importance to fermentation tests in diagnosis.

In summing up the results of our studies we wish to call attention to the following points:

Pneumococci may acquire certain unusual cultural properties in human blood or in human exudates. The power to ferment inulin may become lost, and the ability to cause precipitation in glucose-serum-agar may be gained. In other words a substitution of the common cultural features of pyogenic streptococci may take place; the organisms then resemble the "streptococcus cultural type."

Although many strains fail to cause hemolysis in blood agar, the organisms isolated from the circulating blood frequently cause hemolysis when grown in the blood of the host.

Pneumococci in the blood of a patient suffering from a general infection with this organism may differ in cultural characteristics from those obtained from the metastatic foci.

The cultural properties of pneumococci derived from the same source and isolated at different periods in the course of the patient's disease may vary, for at one time we may see the typical pneumococcus morphology associated with the streptococcus cultural type, at another, we may note typical pneumococcus morphology and pneumococcus cultural features.

Pneumococci of the streptococcus cultural type may be possessed either of the morphology of typical pneumococci or of ordinary streptococci.

It is possible to convert some but not all strains possessing the streptococcus cultural type into typical pneumococci by inoculation into white mice. All of the pneumococci isolated from one case may not permit of this change.

The morphology of pneumococci as it appears with the capsule stain proposed by one of us[1] may alone give a clew in the establishment of a diagnosis. By means of the capsule stain we were sometimes able to make a diagnosis several weeks before other substantiating evidence could be obtained. Thus one strain (from a metastatic focus in a case of pneumococcemia), which was diagnosticated as pneumococcus because of its typical morphology (Observation 1) was found to be possessed of streptococcus cultural features; and failed to permit of conversion to the normal type by animal inoculation. In still another instance (Observation 3) where we were dealing with an organism from a case of acute osteomyelitis, the morphology was that of a typical pneumococcus. All the other features, however, were of the streptococcus type and animal inoculations proved useless. Here later cultures proved conclusively that the organism in question was pneumococcus.

The tendency of pneumococci of the streptococcus cultural type as well as those which have been converted to the normal variety, seems to be toward a gradual degeneration which manifests itself in the assumption of *permanent* streptococcic features. Such organisms can then no longer be differentiated from streptococci.

For purposes of diagnosis we deem the following conclusions worthy of consideration:

1. The fermentation of inulin is of limited value in the differential diagnosis between pneumococci and streptococci. Pneumococci may lose the power to produce acid in this carbohydrate either temporarily or permanently.
2. The precipitation of glucose-serum-agar although more frequently a property of streptococci, may also be produced by the action of pneumococci.
3. Hemolysis in blood agar may be caused by both streptococci and pneumococci.
4. The morphology of pneumococci as it appears with the capsule stain employed by us frequently allows a diagnosis to be made when

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[1] *Centralbl. f. Bakt.*, 1905, 39, pp. 216.

all other means fail. Whenever an organism presents the typical pneumococcus capsule together with the cultural features of a streptococcus, we must have recourse to animal inoculation with a view to converting the organisms into the normal variety. When this fails, the diagnosis, although not absolutely certain, may, according to our experience, be safely made in favor of the pneumococcus.